REMARKS/ARGUMENTS

Status of the Application

Claim 26 has been currently amended to recite at least 90% sequence identity.

Claims 27 and 28 have been currently canceled without prejudice to or disclaimer of the subject matter recited therein.

Claims 26 and 29-40 are now pending, with claim 26 being the sole independent claim.

Rejections Under 35 U.S.C. § 101

Claims 26-40 have been rejected under 35 U.S.C. § 101 as not being supported by either a specific and/or substantial assorted utility or a well-established utility.

The claims as currently amended are drawn to an isolated polynucleotide encoding a polypeptide with diacylglycerol acyltransferase (DGAT) activity and at least 90% amino acid sequence identity to SEQ ID NO:16, compositions comprising said isolated polynucleotide, and related methods. The specification describes amino acid sequence homology of SEQ ID NO:16 with known and putative DGAT proteins at pages 20-22 and Figures 1A-1C. The specification also notes on page 29 that assays for DGAT activity are presented by M. Andersson et al. (1994) J. Lipid Res. 35:535-545.

Applicants submit concurrently herewith a Declaration under 37 C.F.R. § 1.132. This Declaration describes experiments conducted with the protein-coding regions present on soybean cDNA clone sr1.pk0098.a8 (SEQ ID NOs:15 & 16) and wheat cDNA clone wr1.pk0119.b6:fis (SEQ ID NOs:21 & 22). As shown in the Declaration, the soybean protein encoded by sr1.pk0098.a8 and the wheat protein encoded by wr1.pk0119.b6:fis were each found to have DGAT activity. Applicants respectfully submit that these experimental results establish a specific and substantial utility for the claimed inventions.

Applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 101.

Rejections Under 35 U.S.C. § 112, 1st Paragraph

Written Description

Claims 26-40 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

Currently amended claim 26 is drawn to an isolated polynucleotide encoding a polypeptide having diacylglycerol acyltransferase (DGAT) activity and at least 90% sequence identity to SEQ ID NO:16. Dependent claims 29-40 also require the isolated polynucleotide to encode a polypeptide having DGAT activity.

At the filing date of the first provisional application to which this application claims priority, there was a known mouse DGAT protein sequence (GI No. 3859934; SEQ ID NO:25), a putative human DGAT sequence (GI No. 3746533; page 20, line 2 of the specification) and a putative *Arabidopsis thaliana* DGAT sequence (GI No. 5050913; SEQ ID NO:26). Subsequently, U.S. Patent No. 6,100,077, "Isolation of a gene encoding diacylglycerol acyltransferase", concerning the human DGAT gene, issued on Aug. 8, 2000, and a journal publication verified the DGAT activity of the *Arabidopsis* protein (Zou et al. (1999) "The *Arabidopsis thaliana* TAG1 mutant has a mutation in a diacylglycerol acyltransferase gene" *Plant J. 19*:645-653). These references were presented in the IDS of the instant application.

Appendix Ia-Id[1] presents an alignment of amino acid sequences for the mouse, human and *Arabidopsis* DGAT proteins from the literature and the DGAT sequences in the specification for *Arabidopsis* (SEQ ID NO:2), rice (SEQ ID NO:14), soybean (SEQ ID NO:16) and wheat (SEQ ID NO:22). The human DGAT sequence was present in Oelkers et al. (1998), "Characterization of Two Human Genes Encoding Acyl Coenzyme A:Cholesterol Acyltransferase-related Enzymes", J. Biol. Chem. 273:26765-26771 (previously presented in the IDS of the instant application). The human DGAT gene was found because of its sequence similarity to acyl-CoA:cholesterol acyltransferase (ACAT) genes, and was initially called ACAT-related gene product 1 (ARGP1). From page 26769 of Oelkers et al., "[t]he predicted ARGP1 protein displays 28% identity with ACAT1 over this portion of the

[1] In Appendix Ia-Id, the numbering of the consensus sequence is given below the sequences, and the numbering of individual sequences is given to the left of each row of sequence, and to the right of the final row of sequence. Bases that are identical in all genes at a given position are indicated by an asterisk.

molecule and includes a FY.DWWN motif present in all cloned ACATs and shown to be important for enzymatic activity (Fig. 7A)."

The FYXDWWN motif, where X is any amino acid, is shown in Appendix Ia-Id as "Domain A" and is totally conserved for all the DGAT sequences presented from human, mouse and four plant species. In Figure 7 of Oelkers et al., attention is also drawn to a conserved serine at position 210, which they cite as critical to the activity and stability of Chinese hamster ovary ACAT1. This serine is conserved in all the DGAT sequences presented in Appendix Ia-Id. As an acyltransferase, it is not surprising for DGAT to have some structural features in common with ACAT proteins.

One reason that the human ARGP1 protein was initially identified as a DGAT protein was the presence of a candidate diacylglycerol-binding domain, HX[FWY]XX[KR]XFXXP (where X is any amino acid), presented in Figure 7A of Oelkers et al. This motif was derived from the diacylglycerol-binding sites of protein kinase C and diacylglycerol kinases. This binding domain is totally conserved in the human and mouse DGAT sequences, and highly conserved in the plant DGAT proteins (in plants, isoleucine (I) or leucine (L) replaces phenylalanine (F) at position eight in this region). Additionally, for the two mammalian and five plant DGAT sequences presented in Appendix Ia-Id, there is a consensus sequence of HKWXXRHXYXP (Domain B), which is highly similar, and less variable, than the consensus sequence presented by Oelkers et al. The diacylglycerol-binding domain is also mentioned in the discussion of the amino acid sequence of Arabidopsis DGAT in Zou et al. Additionally, Zou et al. cite the presence of an invariant proline at position 224 that may participate in acyl-CoA binding. This invariant proline is present in all the DGAT sequences presented in Appendix Ia-Id.

Oelkers et al. describe the presence of a potential N-linked glycosylation site (NLT), and a putative tyrosine phosphorylation motif (KPFKDMDY). These two motifs are conserved among the mammalian DGATs, but not among the plant DGAT proteins (Appendix Ia-Id; shaded regions). Zou et al. also noted the lack of a putative tyrosine phosphorylation motif in the *Arabidopsis* DGAT protein, but identified a consensus sequence (X-L^{200}-X-K^{202}-X-X-S^{205}-X-X-X-V^{209}) that is a targeting motif typical of members of the SnRK1 protein kinase family (Appendix Ia-Id; shaded region of SEQ26_Arab). This consensus sequence is not conserved

among the other plant DGAT proteins. Applicants maintain that the disclosure of multiple plant DGAT amino acid sequences in the instant application assist one skilled in the art in the identification of conserved domains.

In view of the structural and physical characteristics cited above and the reduced breath of the currently amended claims, Applicants respectfully request withdrawal of the rejection of claims 26 and 29-40 under 35 U.S.C. § 112, first paragraph, written description.

Enablement

Claims 26-40 were also rejected under 35 U.S.C. §112, first paragraph, for lack of enablement, since the claimed invention is not supported by either a specific and/or substantial asserted utility or a well established utility.

Applicants respectfully submit, for reasons cited above regarding the rejection under 35 U.S.C. § 101, that there is a specific and substantial utility for the claimed inventions.

Claims 26-40 were also rejected for lack of enablement for sequences that encode a polypeptide with at least 80%/85%/90%/95% sequence identity to SEQ ID NO:16, for lack of guidance as to which amino acid changes could be made and still produce a functional enzyme.

For reasons cited above, Applicants submit that the application has enabled one skilled in the art to make and/or use the invention. The currently amended claims are limited to polynucleotides that encode a polypeptide having DGAT activity and at least 90% sequence identity to SEQ ID NO:16. A reference for a DGAT assay is provided on page 29, lines 4-6. Applicants note that SEQ ID NO:16 has 65.9% sequence identity to the *Arabidopsis* DGAT protein (Appendix II), which has been confirmed as having DGAT activity by Zou et al. Additionally, SEQ ID NO:16 has the conserved Domains A and B, and the conserved amino acids Serine-210 and Proline-224 (Appendix Ia-Id). Appendix II presents the percent sequence homologies for each pair of sequence among the DGAT proteins in the alignment of Appendix Ia-Id. The highest percent homology is between the human and mouse proteins (84.4%). The *Arabidopsis* DGAT protein has 31.6 and 31.7% sequence identity with the human and mouse DGAT proteins, respectively. Similar to *Arabidopsis*, the soybean protein (SEQ ID NO:16) has 30.9% sequence identity with

the human and mouse proteins. Additionally, the soybean protein has a range of 58.6 to 65.9% sequence identity with the corresponding proteins from rice, wheat and *Arabidopsis*.

Knowledge about conserved domains and amino acid residues, present in sequences from the art and the specification, provide specific guidance to one of ordinary skill as to which structures are likely to be necessary for enzyme activity. Molecular biological techniques are available to make changes in the sequence of SEQ ID NO:16 that would not eliminate enzyme activity. For example, the specification at page 6, line 31, and continuing through page 7, line 5, discloses alterations in nucleotide sequence that are not expected to alter functionality, such as alterations that produce a chemically equivalent amino acid at a given site or alterations in the N- or C-terminal portions. As presented in Examples 7 and 8, these genes can be expressed in a heterologous organism, such as *E. coli*, and assayed for enzyme activity in vitro according to methods known in the art, such as the method of M. Andersson et al. (1994) *J. Lipid Res. 35*:535-545 (reference cited in Example 8).

Page 1 of the specification, lines 11-14, describes that triacylglycerols are quantitatively the most important storage form of energy in eukaryotic cells and that DGAT catalyzes the only committed step in triacylglycerol biosynthesis. Plant genes containing DGAT activity would be expected to be useful in modification of the oil content of transgenic plants. The Office Action asserts that modifying plant biosynthetic pathways by transforming plants with genes encoding enzymes involved in said pathway is highly unpredictable. Applicants note that previous characterization of the EMS-induced *Arabidopsis* mutant AS11 (Katavic et al. (1995) *Plant Physiol. 108*:399-409; previously presented in the IDS) showed a reduced oil content phenotype, and this mutation was found to be in the DGAT gene (SEQ ID NO:26; Zou et al. (1999) *Plant J. 19*:645-653; previously presented in the IDS). Consequently, overexpression of DGAT would be one approach for attempting to increase oil levels in transgenic plants. Applicants note that this has been successfully demonstrated for tobacco transformed with the *Arabidopsis* DGAT gene (Bouvier-Nave et al. (2000) *Eur. J. Biochem. 267*:85-96; previously presented in the IDS).

In view of the foregoing, Applicants respectfully request withdrawal of the Section 112, 1st paragraph, enablement rejection.

Summary

In view of the foregoing amendments and remarks, Applicants submit that this application is in condition for allowance. In order to expedite disposition of this case, the Examiner is invited to contact Applicants' representative at the telephone number below to resolve any remaining issues. Should there be a fee due which is not accounted for, please charge such fee to Deposit Account No. 501447 (Potter Anderson & Corroon LLP).

Respectfully submitted,

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